

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Viral PCR and serologies

PCR testing for viral pathogens was performed on a nasopharyngeal swab (SARS-CoV-2, respiratory virus panel) and serum (enterovirus panel, parvovirus). We did not detect any evidence of viral infection with these assays. We also did not detect antibodies against HIV and SARS-CoV-2 nucleocapsid protein. Data were most complete for Patient 1 and are included in Supplementary Table 1.

Serological examination for viruses associated with myocarditis is of limited value due to the inability to distinguish past from active infection and high prevalence of positive testing in healthy individuals. We performed PCR assays for viruses associated with myocarditis on nasopharyngeal secretions and serum, which were negative. Standardized assays to detect viral genomes within myocardial tissue (next generation sequencing, immunostaining, *in situ* hybridization) are not available at our institution. We recognize this limitation and cannot completely rule out a viral etiology. We also performed serological testing that identified a Coxsackie B type 4 antibody. This test is of minimal clinical value as it cannot distinguish between prior exposure and active infection. In addition, the population prevalence of these antibodies is high (1-2). PCR assays for this virus (nasopharyngeal swab and serum) were negative, and in conjunction with lack of patient-reported viral prodrome, suggested that active infection was unlikely.

Autopsy, Patient 2

The autopsy was performed with complete gross examination with limited tissue sampling for microscopic examination (heart, kidney and brain only). The gross examination of the heart showed glistening pericardial surface without evidence of pericardial effusion or pericarditis. Both right and left coronary arteries and their major branches showed minimal atherosclerosis

without evidence of luminal narrowing or thromboemboli. The gross examination of the myocardium showed no evidence of scars/prior infarct. Scattered areas of subtle mottling were noted in both ventricles, and tissue from those areas were submitted for microscopic examination. In total, two pieces of tissue from the right ventricle, two pieces of tissue from the left ventricle, and one piece of tissue from the interventricular septum were microscopically examined, and all pieces contained inflammatory infiltrates associated with cardiomyocyte damage, consistent with myocarditis. The inflammation spanned the full thickness of the cardiac tissue, with a tendency of more dense inflammation and cardiomyocyte damage towards the epicardium (Supplemental Figure 4). The degree and distribution of inflammation and cardiomyocyte damage were similar across different anatomic sites. We noticed that samples from the right ventricle contained more eosinophils compared to the other two anatomic sites. Abundant T-cells (CD3, CD4, CD8) and macrophages (CD68) admixed with B-cells (CD19) were observed. These findings were similar to Patient 1 (main figure), with less plasma cells (CD138) present (Supplemental Figure 5). A piece of right atrium was sampled for microscopic examination and showed no evidence of myocarditis. The small arterioles and endothelial cells examined microscopically showed no evidence of vasculitis/endotheliolitis or microthrombi. While a differential diagnosis of eosinophilic myocarditis was considered, the eosinophils were not the predominant cell type within the inflammatory infiltrate in both patients. However, we cannot entirely exclude the possibility that the minor population of eosinophils we observed were associated with certain medication as a contributory factor.

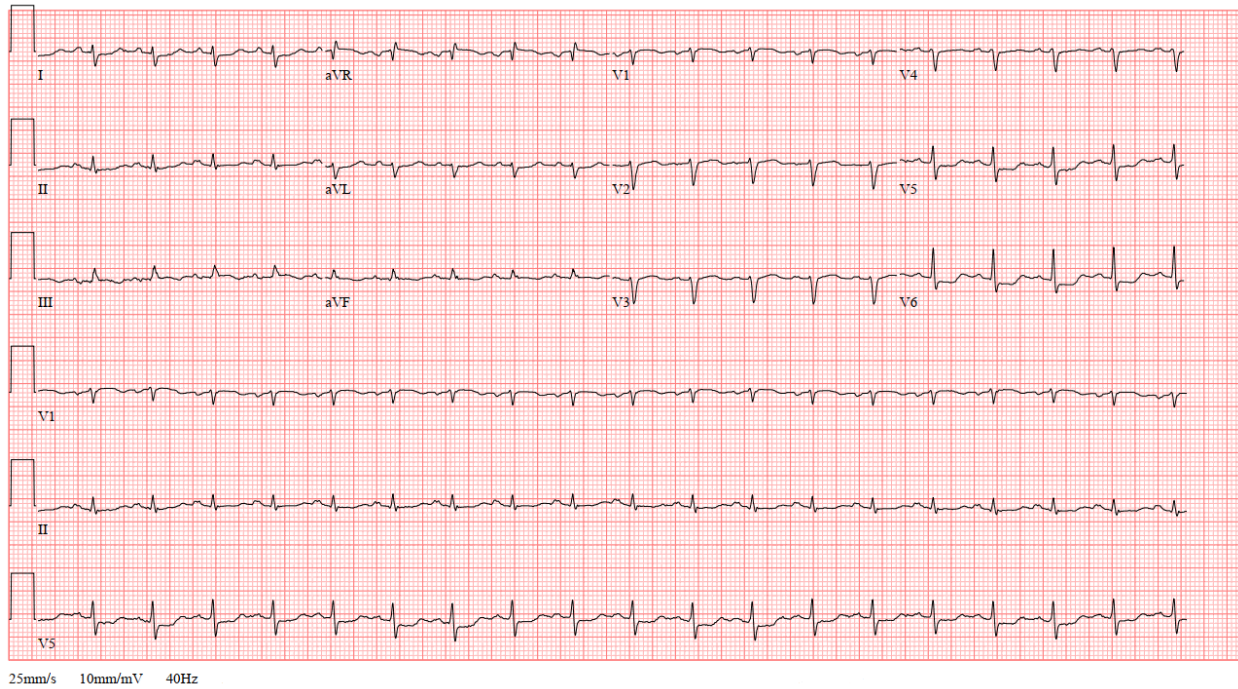
The bilateral lungs examined grossly showed no evidence of pulmonary embolism, pneumonia, consolidation, infarction or any specific pathologic etiology. The gastrointestinal system and hepatopancreatobiliary system were examined grossly without significant findings.

The gross and microscopic examination of bilateral kidneys showed no significant pathological findings. Incidental benign renomedullary interstitial cell tumors were noted microscopically. The brain and spinal cord were examined grossly and microscopically and showed no evidence of structural abnormalities, hemorrhage, degenerative changes, infarction/ischemia or vasculopathy.

Additional References

1. Miller NA, Carmichael HA, Calder BD, et al. Antibody to Coxsackie B virus in diagnosing postviral fatigue syndrome. *British Medical Journal* 1991; 302: 140-3.
2. Bell EJ, McCartney RA. A study of Coxsackie B virus infections, 1972-1983. *J Hyg (Lond)* 1984; 93: 197-203.
3. Larsen KF, Ammirati E, Adler ED, et al. Myocarditis after BNT162b2 and mRNA-1273 Vaccination. *Circulation*. 2021. Advance online publication.
4. Muthukumar A, Narasimhan M, Li Q, et al. In Depth Evaluation of a Case of Presumed Myocarditis Following the Second Dose of COVID-19 mRNA Vaccine. *Circulation*. 2021. Advance online publication.
5. Rosner CM, Genovese L, Tehrani BN, et al. Myocarditis Temporally Associated with COVID-19 Vaccination. *Circulation*. 2021. Advance online publication.

Patient 1.



Patient 2.

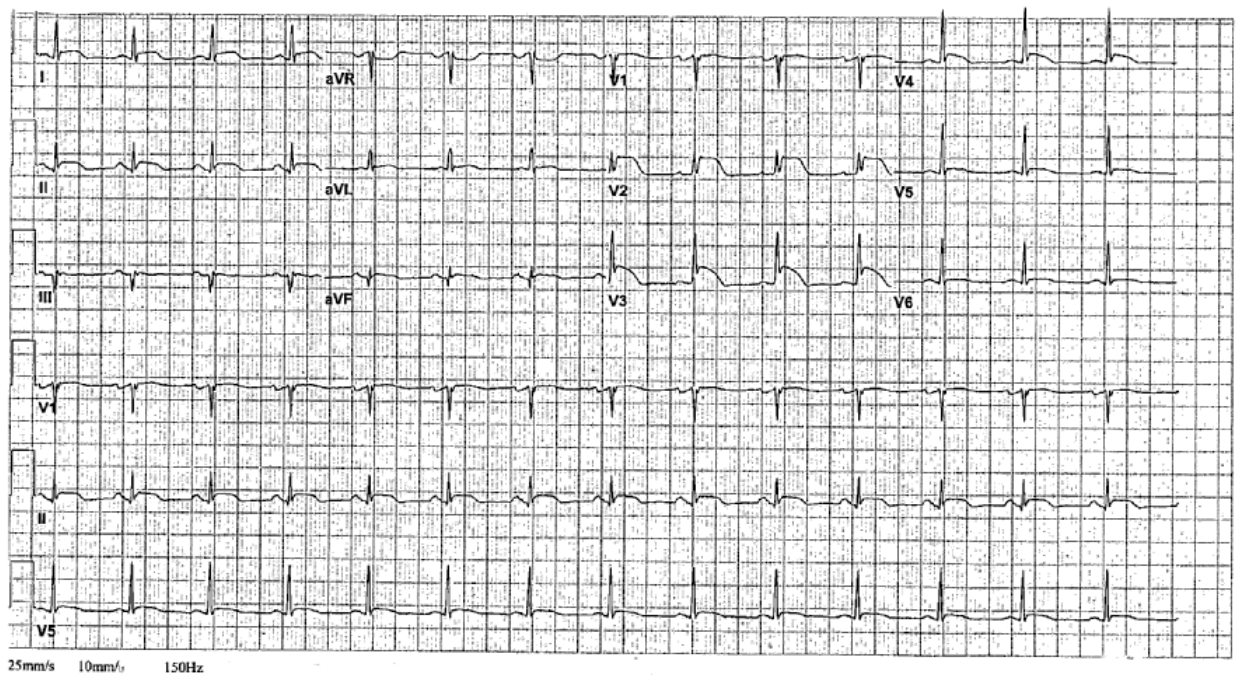


Figure S1: Electrocardiograms (EKG) on presentation.

EKGs of Patient 1 and 2 on presentation. EKG for patient 1 shows sinus tachycardia with low voltage and ST segment depression, most notably in the lateral leads. EKG for patient 2 shows normal sinus rhythm with diffuse ST segment elevation.

Patient 1, endomyocardial biopsy

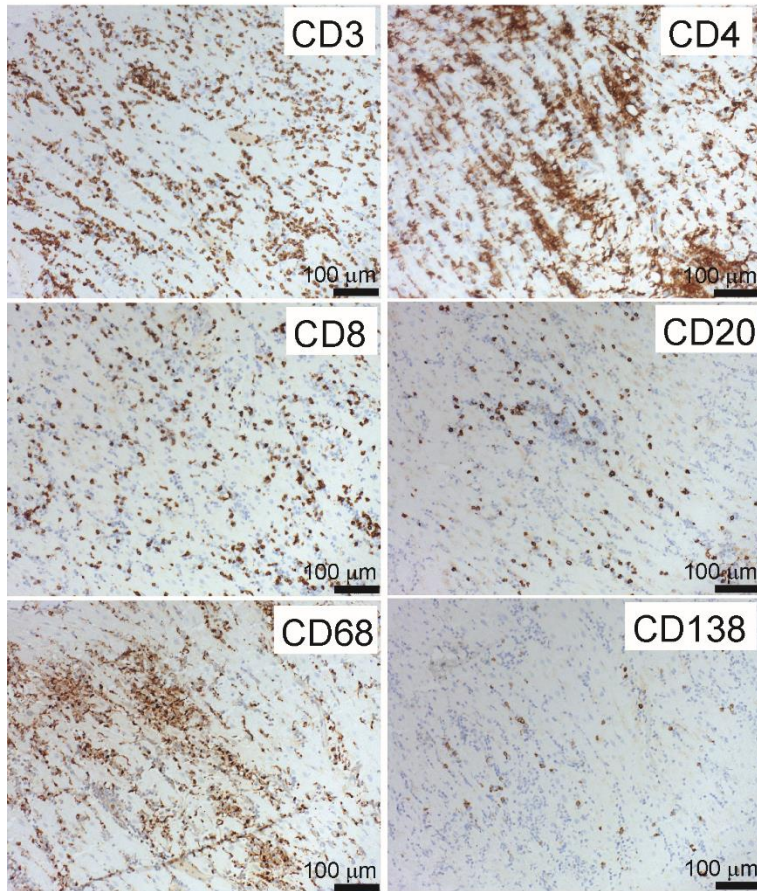


Figure S2: Immunohistochemistry from Patient 1. A panel of immunohistochemistry stains were performed to characterize the immune cells. The CD3 stain highlighted abundant amount of T-cells. The CD4 stain highlighted CD4+ helper T cells as well as macrophages. The CD8 stain highlighted CD8+ cytotoxic T-cells. The CD20 stain highlighted scattered B-cells. The CD68 stain highlighted abundant amount of macrophages. The CD138 stain highlighted rare plasma cells. The immunohistochemistry stained images were taken with 10X eyepieces and a 20X objective.

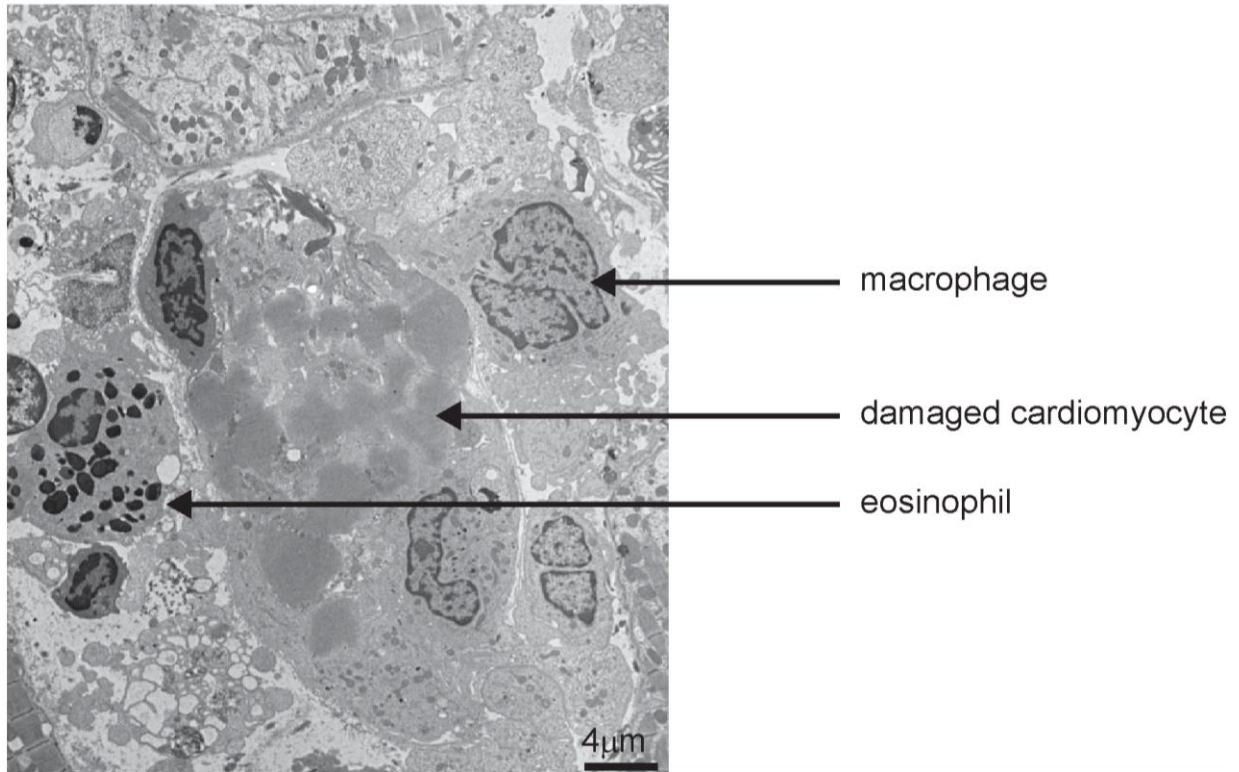


Figure S3: Representative electron microscope (EM) image from Patient 1. The EM study showed a damaged cardiomyocyte with loss of normal sarcomeric ultrastructures and surrounded by mixed inflammatory cells. The scale bar is provided at the right lower corner. The magnification is 4000x.

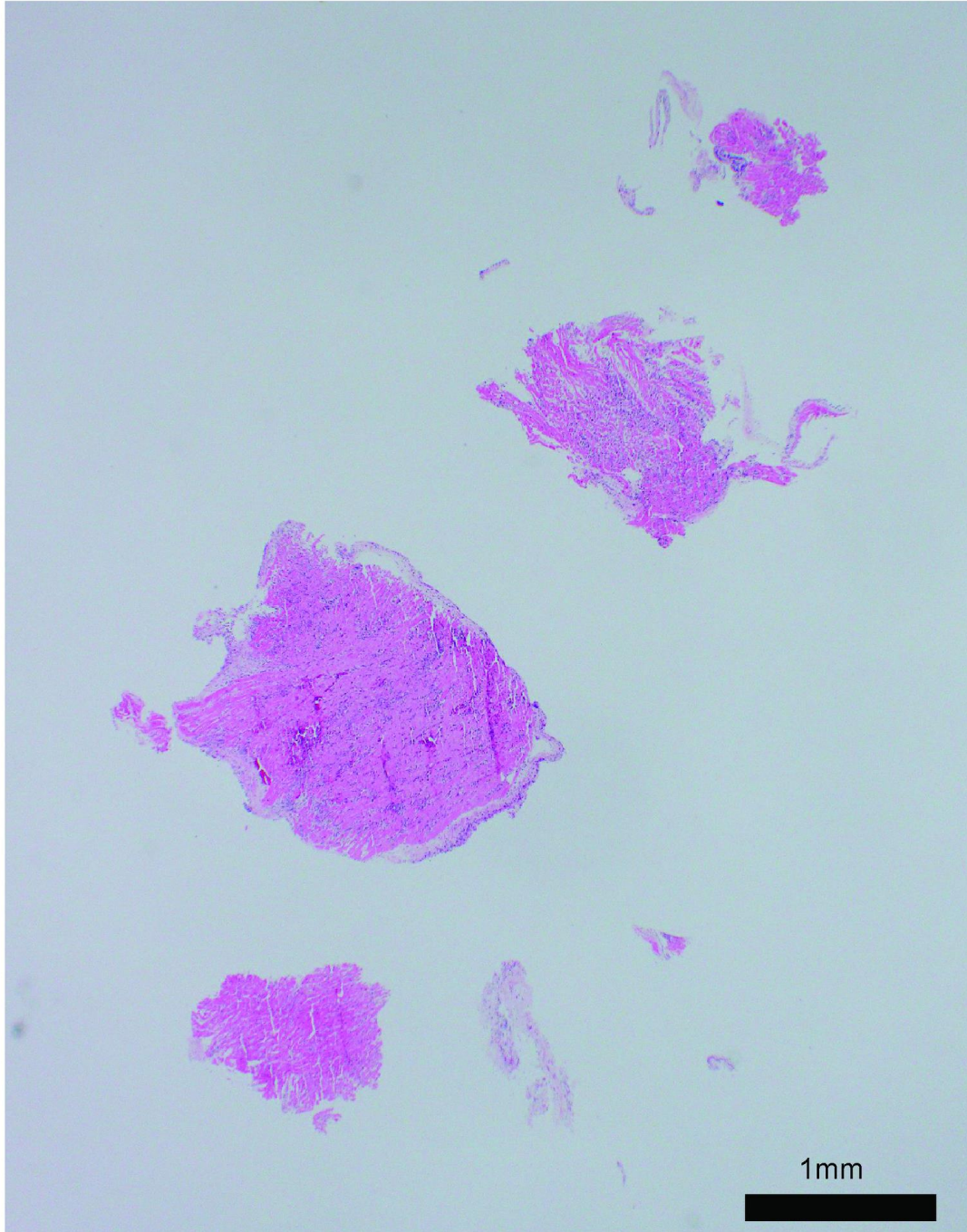
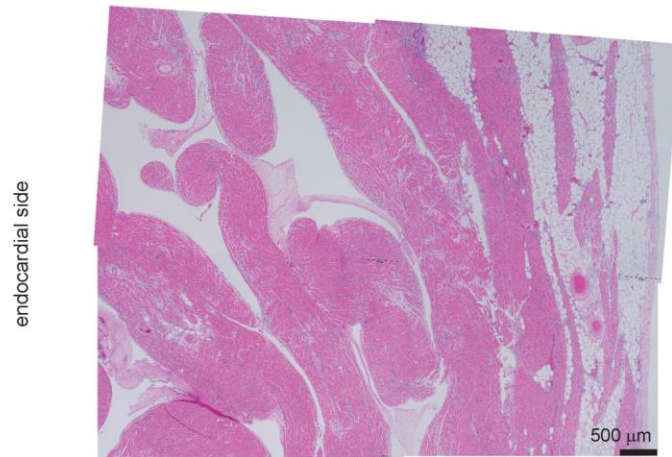


Figure S4: Low power view of HE stained slides of the biopsy from Patient 1. In total, four pieces of tissue were examined, and all four pieces were affected. The left lower corner piece does not show definite myocardial necrosis, but with inflammatory infiltrates. This picture was taken with 10X eyepieces and a 2X objective lens.

A Patient 2: Right ventricle



B Patient 2: left ventricle

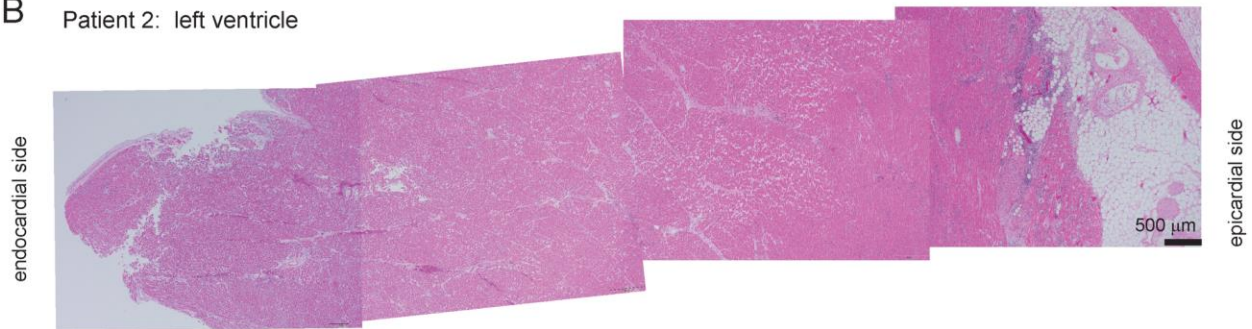
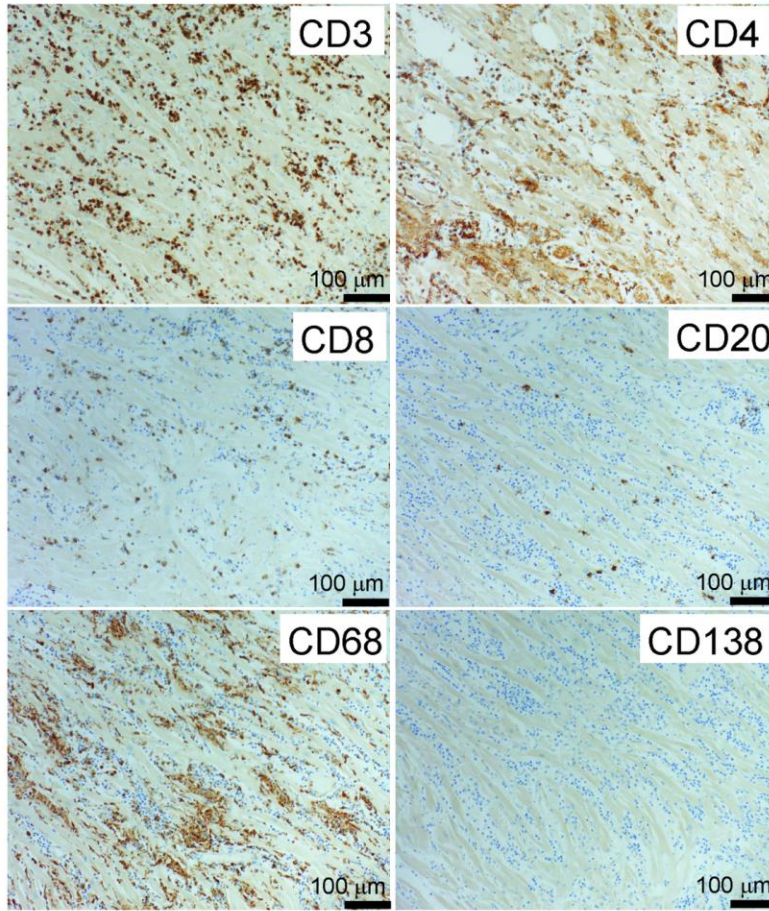
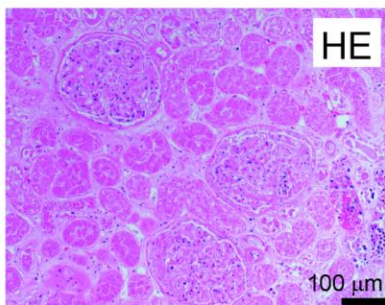


Figure S5: Composite HE stained images of full-thickness cardiac tissue of Patient 2. A, right ventricle. **B,** left ventricle. Multiple low-power images were taken (10X eyepieces and a 4x objective lens) to construct these composite images.

A Patient 2, right ventricle



B Patient 2, kidney



C Patient 2, brain

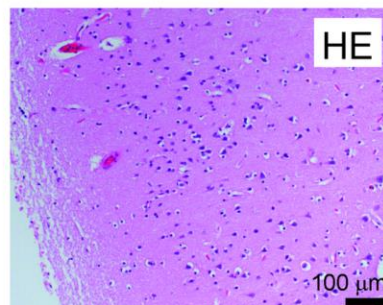


Figure S6: Additional histological findings of Patient 2. **A**, Immunohistochemistry stains for CD3, CD4, CD8, CD20, CD68, and CD138 were performed using right ventricle tissue. Abundant T-cells (CD3, CD4, CD8) and macrophages (CD68) admixed with B-cells (CD19) was observed. These findings were similar to Patient 1 (Figure S2), with less plasma cells (CD138) present. **B-C**, H&E staining of kidney and brain without evidence of pathology.

Table S1. Clinical and Laboratory Data

	Patient 1	Patient 2
General characteristics		
Age	45	42
Sex	Female	Male
Race	Asian American	African American
Admission Vital Signs		
Heart rate (beats per minute)	110	96
Respiratory rate (per minute)	18	16
Blood pressure (mmHg)	92/52	131/62
Temperature (°C)	36.8	38
Pulse oximeter saturation (%)	98% (room air)	100% (room air)
Body Mass Index (kg/m ²)	29.8	27.3
Admission Laboratories		
WBC	6.5	8.8
%neutrophils	54.2	69.9
%lymphocytes	34.4	14.5
%eosinophils	3.4	0
Hemoglobin (g/dL)	12.5	14.8
Hematocrit	37.4	44.7
Platelets	350	196
Sodium (mmol/L)	136	136
Potassium (mmol/L)	3.7	4.4
Chloride (mmol/L)	102	99
Carbon dioxide (mmol/L)	22	25
BUN (mg/dL)	10	26
Creatinine (mg/dL)	0.5	1.5
AST (U/L)	71	199
ALT (U/L)	35	45
Total bilirubin (mg/dL)	0.2	0.6
Alkaline phosphatase (U/L)	67	60
Troponin I (ng/mL) on presentation	6.140 (reference 0-0.30 ng/mL)	27.0 (reference 0.012-0.120 ng/mL)
Other Labs		
Peak Troponin I	10,453 (high sensitivity assay, reference ≤ 17 ng/L)	44.30 (reference 0.012-0.120 ng/mL)
NT proBNP (reference <300 pg/mL)	Not available	6260
Lactate (reference 0.5-2.2 mmol/L)	1.3	Not available
CRP (reference <1.0mg/L)	26.8	Not available
ESR (reference 1-20 mm/hr)	38	6
ANA	Negative	Not available
TSH (reference 0.30-4.20 mIU/mL)	1.15	1.20
Viral evaluation		
SARS-CoV-2 PCR (nasopharyngeal)	Negative	Negative
Respiratory viral panel (nasopharyngeal swab) Influenza A/B Rhinovirus/Enterovirus Coronavirus (229E,	Negative	Not available

HKU1, NL63, OC43) Adenovirus Metapneumovirus Parainfluenza 1-4 B. pertussis B. parapertussis C. pneumoniae M. pneumoniae		
HIV 1/2 Antibody/p24 Antigen	Nonreactive	Not available
Parvovirus B19 PCR (serum)	Not detected	Not available
Enterovirus PCR (serum)	Negative	Not available
SARS-CoV-2 nucleocapsid IgG	Nonreactive	Not available
Enterovirus Antibodies Coxsackie A, serotype 9 Coxsackie B Virus, Ab type 1 Coxsackie B Virus, Ab type 2 Coxsackie B Virus, Ab type 3 Coxsackie B Virus, Ab type 4 Coxsackie B Virus, Ab type 5 Coxsackie B Virus, Ab type 6 Echovirus, Ab type 6 Echovirus, Ab type 7 Echovirus, Ab type 9 Echovirus, Ab type. 11 Echovirus, Ab type 30	<1:8 1:10 <1:10 <1:10 1:320* <1:10 <1:10 <1:10 <1:10 <1:10 1:10 <1:10	Not available
Medical History	Overweight	Active tobacco use
Medications prior to illness	None	None
Medications received during illness/hospitalization	Azithromycin Ceftriaxone Fentanyl Fluticasone Furosemide Heparin Midazolam Milrinone Potassium Chloride	Aspirin Amiodarone Amoxicillin Benzonatate Colchicine Epinephrine Fentanyl Furosemide Heparin Indomethacin Ketamine Lidocaine Magnesium sulfate Metoclopramide Midazolam Morphine sulfate Norepinephrine Ondansetron Promethazine Sodium bicarbonate

Cardiac Diagnostic Testing		
ECG	Sinus tachycardia, ventricular rate of 115bpm, low voltage, lateral ST segment depression	Sinus rhythm, ventricular rate of 84bpm, diffuse ST elevations
Transthoracic echocardiogram	Severe global LV hypokinesis LVEF 15-20% Normal LV size (LVIDd 4.4cm) No LVH Normal RV systolic function Normal atria No AR No MR No PI Trace TR Grade I diastolic dysfunction Dilated IVC Small pericardial effusion	Severe global LV hypokinesis LVEF 15% Normal LV size (LVIDd 5.1cm) LVH (LVPWd 1.5cm, IVSd 1.8cm) RV systolic dysfunction Normal atria Trivial AR Mild to moderate MR Mild PI Moderate TR Grade I diastolic dysfunction Trivial pericardial effusion
Cardiac MRI	Delayed subepicardial enhancement of the LV lateral wall and overlying pericardial enhancement	Not available
Coronary angiography	Right dominant system No angiographic evidence of CAD	Left dominant system No angiographic evidence of CAD LV 83/13 mmHg, Aortic 82/61 mmHg
Hemodynamics/right heart catheterization	RA 17mmHg RV 40/13 mmHg PA 40/25mmHg, mean 30mmHg PCWP 25mmHg Ao saturation 93% PA saturation 46% Cardiac output (Fick) 2.85 L/min Cardiac index (Fick) 1.66 L/min/m ²	Not available

* In the absence of positive PCR testing, detectable neutralizing antibody titer is consistent with prior exposure or infection

Abbreviations: LV left ventricle, LVH left ventricular hypertrophy, RV right ventricle, AR atrial regurgitation, MR mitral regurgitation, PI pulmonary insufficiency, TR tricuspid regurgitation, IVC inferior vena cava, RA right atrium, PA pulmonary artery, PCWP pulmonary capillary wedge pressure, Ao aortic